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④ Furano[2,3-F]isoindoles as aldose reductase inhibitors.

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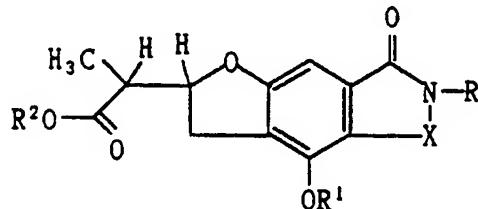
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Description

The present invention relates to an aldose reductase inhibitor and a method for producing the same. The compound of the invention is represented by the formula:

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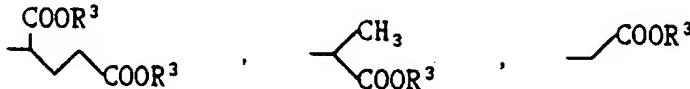


15

wherein X is methylene or carbonyl;
R is

20

25

or R⁴;R¹, R², R³, and R⁴ are each hydrogen or C₁-C₄ alkyl.

The incidence rate of diabetes has hitherto increased and various complications thereof have become a quite serious problem. The complications of diabetes may be caused by, for example, accumulation of polyol (e.g., sorbitol), free radical peroxidation, and glycosylation of proteins at the site of lysine residues. An inhibitor for aldose reductase (abbreviated hereinafter as AR) relating to polyol accumulation is expected to serve as a medicine for diabetic complications, that is, diseases arising from diabetes such as diabetic neuropathy, diabetic cataract, diabetic keratopathy, diabetic retinopathy, and diabetic nephropathy. Although there have been many investigations into the development of such a medicine, development of more beneficial inhibitors is still necessary (see Reinhard Sages, *Advances in Drug Research*, 18, 139 -175 (1989)). Typical examples of AR inhibitors include Quercitrin reported by Hörhammer et al. (see *Arch Pharm.*, 292, 113 (1959)).

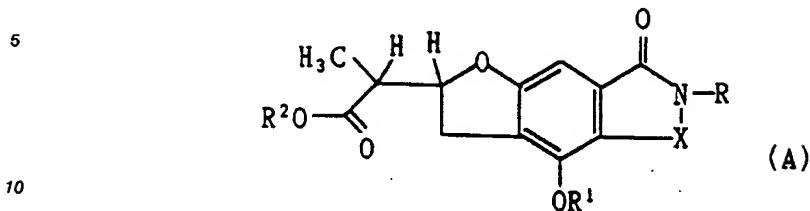
EP-A 0 109 866 discloses sulfonyl urea derivatives which are active as hypoglycemic medicaments in the treatment of diabetes.

The treatment of diabetic complications with compounds which are effective as aldolase reductase inhibitors has been described for heterocyclic oxophthalazinyl acetic acid derivatives (EP-A 0 222 576), 1,2-dihydro-2-oxo (or 2-thioxo)cyclohepta[b]-pyrrole-1-acetic acid derivatives (US-A 4,337,265) and 3-monosubstituted methyl- and 3,3-disubstituted methyl-2-oxo-indoline-1-alkanoic acid compounds (EP-A 0 252 713).

It has been revealed that AR as a rate-limiting enzyme in the metabolic pathway of polyol is present in blood vessels, peripheral nerves, lenses, retinae, and so forth, in which many complications of diabetes often occur. Therefore, the significance of AR became understood in relation to diabetes. In the state of glycophilia such as diabetes, a larger amount of glucose than that of glucose capable of being metabolized in the glycolysis system is present in cells and the metabolism of glucose in the metabolic pathway of polyol can readily be promoted because glucose is used as a substrate for AR. As a result, the abnormal accumulation of sorbitol is further enhanced. Sorbitol is a relatively stable substance; once the cells produce sorbitol, very little extracellular release of the sorbitol is found. The unbalance between the production and metabolism causes the intracellular accumulation of this sugar alcohol. This results in an increase in intracellular osmotic pressure and a lot of water stands in the cells, thereby making it impossible to maintain the normal function of the cells and exhibiting a disorder of the cells. If the AR activity is inhibited, the abnormal intracellular accumulation of sorbitol can be avoided, and there is a great possibility that the normal function of the cells can be maintained. Accordingly, it is the object of the present invention to

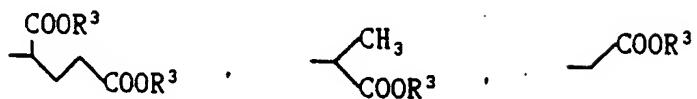
provide compounds having an AR-inhibiting activity.

The object is achieved by a compound of the formula (A):



wherein X is methylene or carbonyl; R is

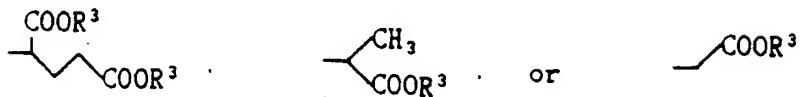
15



or R⁴;

25 R¹, R², R³, and R⁴ are each hydrogen or C₁-C₄ alkyl, or the salt thereof, preferably the compound wherein R is

30

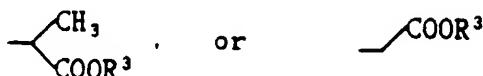


where X is methylene; or

35 R is

R⁴.

40

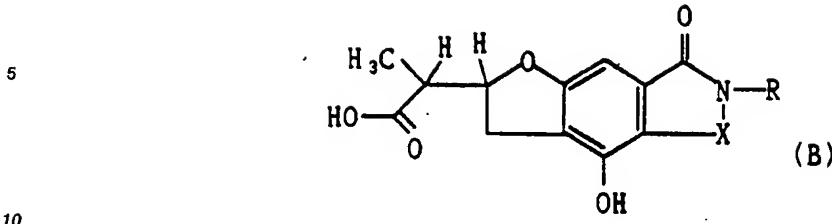


45 where X is carbonyl, or the salt thereof, and more preferably the compound wherein R³ or R⁴ is hydrogen or the salt thereof.

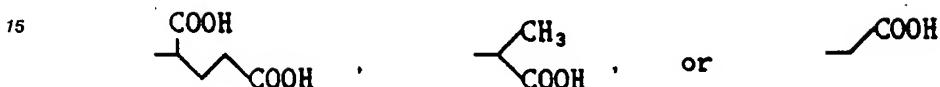
This invention also relates to a method for producing the compound, which comprises culturing Crucibulum sp. RF-3817 or its variants which can produce a compound of the formula(B):

50

55



wherein R is

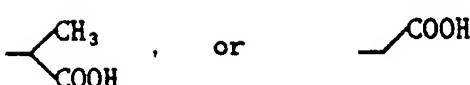


20 where X is methylene; or

R is

H,

25



30

where X is carbonyl;

isolating the above mentioned compound from the cultured medium and optionally alkylating the compound. It further provides a pharmaceutical agent comprising the compound, as an effective ingredient, in association with a pharmaceutically acceptable, substantially nontoxic carrier or excipient, which inhibits an aldose reductase.

In the present invention, the C₁-C₄ alkyl groups include straight- or branched- methyl, ethyl, propyl, and butyl.

The compound of the present invention may be used as a medicine for diabetic complications such as diabetic neuropathy, diabetic cataract, diabetic keratopathy, diabetic retinopathy, and diabetic nephropathy, because of its aldose reductase-inhibiting activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a UV spectrum of RD-01-A₄ of the present invention.

45 Figure 2 is an IR spectrum of RD-01-A₄ of the present invention.

Figure 3 is a ¹H-NMR spectrum of RD-01-A₄ of the present invention.

Figure 4 is a ¹³C-NMR spectrum of RD-01-A₄ of the present invention.

Figure 5 is a diagram showing the results of X-ray analysis of RD-01-A₄ of the present invention.

The strain RF-3817 of the genus Crucibulum capable of producing the compound of the present invention has the following properties.

Various taxonomical properties of the strain RF-3817

The strain RF-3817 was isolated as follows. First, peridioles within the peridium formed on a rotten wood block were immersed in a solution of sodium hypochlorite for one minute, whereby the surface of the peridioles was sterilized. Then, the peridioles were washed with sterile water and the basidiospores formed within the peridioles were placed on an appropriate medium to isolate the strain. On the hyphae of the strain grown on the medium, clamp connections were observed, the width of each hypha being 1 to 5 μ m.

The peridium of the strain formed on the rotten wood block is pyriform, 5 to 8 mm in height, 4 to 6 mm in diameter, and has an ocher color. In the upper portion of the peridium, there is an epiphragm having a pale yellowish-white color, which will dehisce when matured. Within the peridium, lenticular peridioles are observed. The peridioles, 1 to 1.5 mm in size, are attached by funiculi to the inner wall of the peridium.

5 Within the peridioles, there are formed basidiospores, the size thereof being 3 to 5 by 7 to 10 μm .

These properties were compared with the characteristics of each of the genera belonging to the order Nidulariales, the family Nidulariaceae, as described in H.J. Brodie, "The Bird's Nest Fungi," Univ. of Toronto Press, Toronto and Buffalo (1975), and Rokuya Imazeki & Tsugio Hongo, "Colored Illustrations of New Fungus in Japan, Vol. II, (1989)" Hoikusya Publishing Co., Ltd. According to the comparison, the strain 10 of the present invention was identified as a strain of the genus Crucibulum and named Crucibulum sp. RF-3817. The strain of the present invention was deposited on May 9, 1990, under the terms of the Budapest Treaty with the Fermentation Research Institute, Agency of Industrial Science and Technology, 1-3, Higashi 1-chome Tsukuba-shi, Ibaraki-ken 305, and has been assigned "Crucibulum sp. RF-3817" with the Accession No, FERM BP-2888.

15 The following will describe a general method for producing the compound of the present invention.

The production can be performed using a medium composition and conditions which are used in the ordinary production by fermentation. A typical medium usually contains carbon sources, nitrogen sources, inorganic salts, and others. If necessary, vitamins and precursors may also be added to the medium. As the carbon sources, for example, glucose, potato starch, dextrin, glycerol, molasses, and organic acids can be used alone or in a mixture thereof. As the nitrogen sources, for example, soybean powder, corn steep liquor, meat extract, yeast extract, cotton seed powder, peptone, wheat germ, ammonium sulfate, and ammonium nitrate can be used alone or in a mixture thereof. As the inorganic salts, for example, calcium carbonate, sodium chloride, potassium chloride, magnesium sulfate, copper sulfate, manganese chloride, zinc sulfate, cobalt chloride, and various phosphates can be used, and these inorganic salts are added to the medium whenever they are required.

20 The temperature of incubation which can be used is in a range of from about 20 to 40 $^{\circ}\text{C}$ with a temperature of from 25 to 32 $^{\circ}\text{C}$ being preferred. The time of incubation depends greatly upon the scale of fermentation and from about 7 to 10 days are required for mass production. In cases where vigorous foaming occurs during incubation, a defoaming agent such as vegetable oil, lard, or polypropylene glycol 25 can be conveniently added to the medium, before or during the incubation.

30 After culturing, for the purpose of separating the compound of the present invention from the culture, an ordinary process of separating fermentation products is conveniently used. For example, filtration, centrifugation, absorption-elution or chromatography using various ion exchange resins or other active absorbents, and/or extraction using various organic solvents can be adequately combined with each other.

35 The following describes physicochemical characteristics and structures of RD-01-compounds obtained through the above fermentation, incubation, and separation process.

(a) RD-01-A₃

40 State: colorless amorphous powder (acidic and soluble substance in organic solvents such as ethyl acetate, acetone, methanol, and ethanol)

Molecular Formula: C₁₈H₁₉O₉N

Molecular Weight: 393

SIMS:

m/z 394 [M + H]⁺

45 UV Absorption Spectrum:

$\lambda_{\text{max}}^{\text{MeOH}} (\text{E}_{\text{1cm}}^{\infty}) \text{ nm}$

50

MeOH: 215(930), 260(230), 305(70)

0.1N HCl-90% MeOH: 215(770), 260(230), 303(70)

0.1N NaOH-90% MeOH: 275(190), 317(100)

IR Absorption Spectrum:

55

$\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$

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3400, 3182, 2966, 1714, 1653, 1605, 1494, 1452, 1412, 1364, 1270, 1238, 1206, 1067, 978, 941, 769

1H-NMR: (200MHz, d_6 -DMSO) ppm (J =Hz) 1.147(3H, d, J =7.0, CH_3); 1.8 - 2.4(2H, m); 2.235(2H, s); 2.725(1H, qd, J =7.0, 7.0); 2.975(1H, dd, J =7.5, 16.5); 3.2 - 3.4(1H, m); 4.246(2H, s); 4.750(1H, m); 5.020(1H, m); 6.494(1H, s)

5 13 C-NMR: (50MHz, d_6 -DMSO) ppm 11.90(q), 24.13(t), 30.18(t), 30.64(t), 43.86(d), 44.72(t), 53.08(d), 84.22(d), 94.76(d), 117.22(s), 121.88(s), 133.18(s), 148.80(s), 161.22(s), 168.83(s), 172.44(s), 173.87(s), 174.90(s)

10 HPLC

Column: COSMOSIL PACKED COLUMN 5 C₁₈ (4.6φ×150mm)

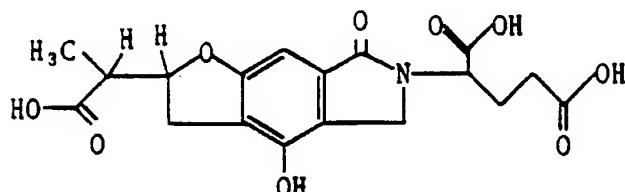
Solvent: acetonitrile : 0.1% TFA = 13 : 87

Flow rate: 1ml/min.

Detection: UV(220 or 254 nm)

Retention time: 12.1 min.

15 Structure:



(b) RD-01-A,

30 State: colorless fine needles (acidic and soluble substance in organic solvents such as ethyl acetate, acetone, methanol, and ethanol)

Molecular Formula: C₁₅H₁₅O₇N

Molecular Weight: 321

35 SIMS:

m/z 322 [M + H]⁺

$[\alpha]_D$: +46.3 ± 1.7°

30 Solubility:

soluble in ethyl acetate, DMSO, methanol, and ethanol; slightly soluble in chloroform and ether; insoluble in petroleum ether.

UV Absorption Spectrum:

40

λ_{max} (E_{1cm}) nm

45 MeOH: 215(810), 258(190), 303(60)

0.01N HCl-90% MeOH: 215(710), 258(190), 303(60)

0.01N NaOH-90% MeOH: 220 sh(630), 275(150), 316(60)

(See Figure 1.)

IR Absorption Spectrum:

50

ν_{max} cm⁻¹

55 3466, 3234, 2918, 1725, 1691, 1634, 1599, 1053, 1459, 1356, 1266, 1235, 1211, 1077, 992, 936

(See Figure 2.)

1 H-NMR: (DMSO) ppm (J =Hz)

1.150(3H, d, $J = 7.0$); 2.717(1H, qd, $J = 7.0, 7.0$); 2.944(1H, dd, $J = 7.2, 16.6$);
 3.310(1H, dd, $J = 9.0, 16.6$); 4.202(2H, s); 4.295(2H, s); 5.001(1H, ddd,
 $J = 7.0, 7.2, 9.0$); 6.492(1H, s)

(See Figure 3.)

5 $^{13}\text{C-NMR}$:

(DMSO) ppm

12.17(q), 30.85(t), 43.67(t), 44.08(d), 47.95(t), 84.28(d), 94.64(d), 117.05(s),
 121.88(s), 133.43(s), 148.93(s), 161.14(s), 168.13(s), 171.09 (s), 174.98(s)

(See Figure 4.)

10 X-ray Analysis:
 HPLC

see Figure 5.

Column: COSMOSIL 5 C₁₈ (20 ϕ × 150mm)

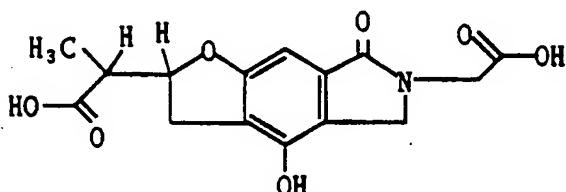
Solvent: acetonitrile : 0.1% TFA = 15 : 85

Flow rate: 9ml/min.

Detection: UV(254 nm)

15 Retention time: 21.4 min.

Structure:



(c) RD-01-A₇

30 State: colorless fine needles (acidic and soluble substance in organic solvents such as ethyl acetate, acetone, methanol, and ethanol)

Molecular Formula: C₁₆H₁₇O₇N

Molecular Weight: 335

35 SIMS: Melting Point: 293 - 296 °C (decomp.)
 m/z 336 [M + H]⁺, m/z 358 [M + Na]⁺, m/z 671 [2M + H]⁺, m/z 693 [2M + Na]

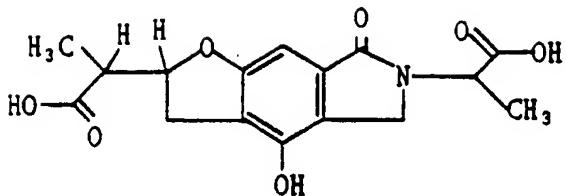
UV Absorption Spectrum: same as that of RD-01-A₃ and RD-01-A₄.
 IR Absorption Spectrum: no data.

40 $^1\text{H-NMR}$: (200MHz, d₆DMSO) ppm ($J = \text{Hz}$) 1.141(3H, d, $J = 7.0$); 1.468(3H, d, $J = 7.4$); 2.711(1H, qd, $J = 7.0, 6.8$); 2.967(1H, dd, $J = 7.4, 16.6$); 3.2 - 3.4(1H, m); 4.243(2H, s); 4.768(1H, q, $J = 7.4$); 5.002(1H, ddd, $J = 6.8, 7.4$, indistinctness); 6.488(1H, s)

45 $^{13}\text{C-NMR}$: (50MHz, d₆DMSO) ppm 12.56(q), 15.75(q), 31.24(t), 44.42(d), 44.92(t), 49.55(d), 84.51(d), 94.79(d), 117.20(s), 122.11(s), 133.71(s), 149.08(s), 161.24(s), 168.06(s), 173.52(s), 174.98(s)

50 HPLC Structure:
 Column: COSMOSIL PACKED COLUMN 5 C₁₈ (4.6 ϕ × 150mm)
 Solvent: acetonitrile : 0.1% TFA = 20 : 80
 Flow rate: 1ml/min.
 Detection: UV(220 or 254 nm)
 Retention time: 6.8 min.

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(d) RD-01-C₁

State: pale yellow amorphous powder (acidic and soluble substance in organic solvents such as ethyl acetate, acetone, methanol, and ethanol)

15 Molecular Formula: C₁₃H₁₁O₆N

SIMS: Molecular Weight: 277

UV Absorption Spectrum: m/z 278 [M + H]⁺, m/z 555 [2M + H]⁺,

20

$\lambda_{\text{max}}^{\text{MeOH}} (E_{1\text{cm}}^{\infty}) \text{ nm}$

25

IR Absorption Spectrum:

¹H-NMR:

MeOH: 223(670), 348(840), 335(110) 0.01N HCl-90% MeOH: 223(580), 348(770), 335(110) 0.01N NaOH-90% MeOH: 234(780), 247sh(580), 393-(150)

30

¹³C-NMR:

no data.

(200MHz, CDCl₃) ppm (J = Hz) (This data is for trimethyl-derivative)*

1.333(3H, d, J = 7.2); 2.825(1H, dq, J = 7.2, 7.2); 3.070(1H, dd, J = 7.6, 16.8); 3.106(3H, s); 3.419(H, dd, J = 9.6, 16.8); 3.719(3H, s); 4.198(3H, s);

5.098(1H, ddd, J = 7.2, 7.6, 9.6); 6.929(H, s)

(50MHz, CDCl₃) ppm (This data is for trimethyl- derivative)*

13.04(q), 23.91(q), 31.70(t), 44.83(d), 52.11(q), 61.65(q), 85.51(d), 100.63(d), 112.44(s), 122.33(s), 136.66(s), 154.64(s) 165.70(s), 166.74(s),

167.72(s), 173.60(s)

35

HPLC

Column: COSMOSIL PACKED COLUMN 5 C₁₈ (4.6φ × 150mm)

Solvent: (acetonitrile : THF = 8 : 2) : 0.1% TFA = 20 : 80

Flow rate: 1ml/min.

Detection: UV(220 or 254 nm)

Retention time: 8.7 min.

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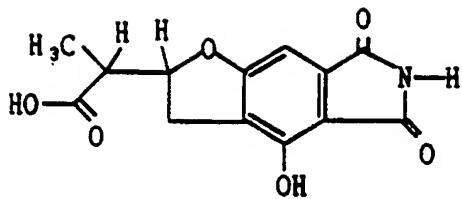
Structure:

45

50

55 *: As the obtained amount of RD-01-C, was very small, the structure of the trimethyl-derivative of RD-01-C, was determined by ¹H-NMR and ¹³C-NMR, then the structure of RD-01-C, was confirmed by SIMS.

*: As the obtained amount of RD-01-C, was very small, the structure of the trimethyl-derivative of RD-01-C, was determined by ¹H-NMR and ¹³C-NMR, then the structure of RD-01-C, was confirmed by SIMS.

(e) RD-01-C₂

15 State: colorless fine needles (acidic and soluble substance in organic solvents such as ethyl acetate, acetone, methanol, and ethanol)
 Molecular Formula: C₁₅H₁₃O₈N
 Molecular Weight: 335

20 Melting point: 246 - 247 °C
 SIMS: m/z 336 [M + H]⁺, m/z 671[2M + H]⁺
 UV Absorption Spectrum:

25 $\lambda_{\text{max}}^{\text{MeOH}} (E_{1\text{cm}}^{1\%}) \text{ nm}$

30 MeOH: 223(600), 247(900), 335(110) 0.01N HCl-90% MeOH: 223(520),
 247(870), 335(110) 0.01N NaOH-90% MeOH: 241(900), 255 sh(600), 277
 sh(60), 395(150)

IR Absorption Spectrum:

$\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$

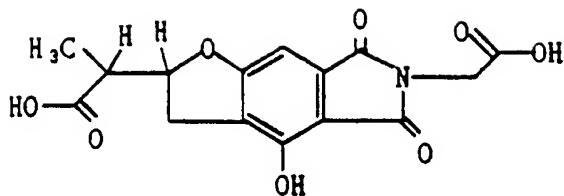
35 3100, 2984, 2948, 2648, 2566, 1696, 1613, 1498, 1457, 1425, 1387, 1358,
 1299, 1277, 1250, 1198, 1115, 1099, 1049, 1020, 994, 935, 865, 823, 803,
 756, 704, 674, 655, 619, 585, 546, 512

40 ¹H-NMR: (200MHz, d₆DMSO) ppm (J=Hz) 1.152(3H, d, J=7.0); 2.798(1H, dq, J=7.0, 7.0); 3.016(1H, dd, J=7.4, 16.8); 3.2 - 3.4(1H, m); 4.190(2H, s); 5.165(1H, ddd, J=7.0, 7.4, indistinctness); 6.775(1H, s)

45 ¹³C-NMR: (50MHz, d₆DMSO) ppm 11.86(q), 30.44(t), ca 38(t), 43.69(d), 85.44(d), 97.49(d), 109.10(s), 119.76(s), 134.78(s), 152.12(s), 165.57(s), 165.69(s), 166.60(s), 169.05(s), 174.21(s)

45 HPLC
 Column: COSMOSIL PACKED COLUMN 5C₁₈ (4.6φ × 150mm)
 Solvent: (acetonitrile : THF = 8 : 2) : 0.1% TFA = 20 : 80
 Flow rate: 1ml/min.
 Detection: UV(220 or 254 nm)
 Retention time: 12.6 min.

50 Structure:



10

(f) RD-01-C₃

15 State: pale yellow oil (acidic and soluble substance in organic solvents such as ethyl acetate, acetone, methanol, and ethanol)

15 Molecular Formula: C₁₆H₁₅O₈N

15 Molecular Weight: 349

15 Melting point: 246 - 247 °C

20 SIMS: m/z 350 [M+H]⁺, m/z 372 [M+Na]⁺, m/z 699 [2M+H]⁺, m/z 721 [2M+Na]⁺

20 UV Absorption Spectrum: same as that of RD-01-C₁ and RD-01-C₂

20 IR Absorption Spectrum: no data

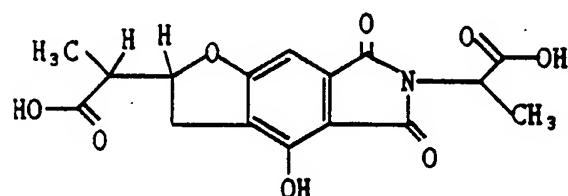
20 ¹H-NMR: (200MHz, d₆DMSO) ppm (J=Hz)
1.143(3H, d, J=7.0); 1.490(3H, d, J=7.3); 2.788(1H, qd, J=7.0, 7.0); 3.006-(1H, dd, J=7.3, 16.6); 3.2 - 3.4(1H, m); 4.739(1H, q, J=7.3); 5.165(1H, ddd, J=7.0, 7.3, indistinctness); 6.746(1H, s)

25 ¹³C-NMR: (50MHz, d₆DMSO) ppm
11.81(q), 14.90(q), 30.46(t), 43.79(d), 46.46(d), 85.61(d), 97.63(d), 109.22(s), 120.02(s), 135.03(s), 152.47(s), 165.98(s), 166.08(s), 166.91 (s), 171.65(s), 174.66(s)

30 HPLC Column: COSMOSIL PACKED COLUMN 5C₁₈ (4.6φ × 150mm)
Solvent: (acetonitrile : THF = 8 : 2) : 0.1% TFA = 22 : 78
Flow rate: 1ml/min.
Detection: UV(220 or 254 nm)
Retention time: 16.0 min.

35 Structure:

40



45

50 Moreover, if necessary, alkylation can be conducted by normal methods to form the compound of the present invention wherein R¹, R², R³, and R⁴ are the same or different from each other.

The following will describe methods for producing these compounds in brief. Compounds of the aforesaid formula (B) are obtained through the above fermentation, incubation and separation process, and among them, the compound wherein R is not hydrogen is named Compound I, the compound wherein R is hydrogen is named Compound i.

55

(1) A mixture of Compound II having hydrogen as R¹ and the same alkyl groups R² and R³ and Compound VIII having hydrogens as R¹ and R² and an alkyl group as R³ in aforesaid formula (A) can be obtained by mixing above Compound I with an alkanol (such as ethanol) and an organic or inorganic acid and by refluxing the mixture for about 1 hour to about 2 hours. The mixture is separated by suitable

means to yield either Compound II or Compound VIII.

Examples of the organic or inorganic acid include hydrochloric acid, sulfuric acid, toluenesulfonic acid, boron trifluoride, and acetic anhydride.

Compound II and Compound VIII are also synthesized by dissolving Compound I in an appropriate solvent such as a tetrahydrofuran(THF)-ether system, adding diazoalkane such as diazomethane, and allowing the solution to stand at room temperature for several minutes to several tens of minutes.

(2) A compound having alkyl groups as R¹, R², and R³ can be obtained by dissolving Compound II in an appropriate solvent such as N,N-dimethylformamide(DMF) and acetone and allowing the solution to react with alkyl iodide such as methyl iodide in the presence of alkali such as potassium carbonate or catalyst such as silver oxide at about 30 °C to about 100 °C for about 30 minutes to about 3 hours.

In this reaction, Compound III having the same alkyl groups R¹, R², and R³ can be synthesized by using alkyl iodide which has the same alkyl as R² and R³ of Compound II, and Compound III' having a different alkyl group R¹ from R² and R³ can be synthesized by using alkyl iodide which has a different alkyl from R² and R³ of Compound II.

Compound III can also be obtained by allowing Compound I to react with alkyl iodide as mentioned above or by dissolving Compound I in an appropriate solvent such as a THF-ether system, adding diazoalkane, and allowing the solution to stand at room temperature for several hours to overnight.

Compound iii having the same alkyl groups R¹, R² and R⁴ can be obtained by treating Compound i with alkyl iodide or diazoalkane as mentioned above.

(3) Compound IV having hydrogen as R³ and the same alkyl groups R¹ and R² can be obtained by partial hydrolysis of esters of Compound IV, which comprises mixing Compound III with several percent to several tens of percent methanol and several tens of percent alkali aqueous solution such as potassium carbonate and stirring at room temperature for several tens of minutes to several hours.

Compound IV' having hydrogen as R³ and different alkyl groups R¹ and R² from each other can be synthesized by partial hydrolysis of esters of Compound III' in the same way as aforesaid.

(4) Compound V having hydrogen as R² and R³ and an alkyl group as R¹ can be obtained by hydrolysis of esters of Compound III, Compound III' or Compound IV, which comprises mixing Compound III, Compound III' or Compound IV with several percents to several tens of percents methanol and several tens of percent potassium carbonate and allowing the mixture to react at 30 °C to 80 °C.

(5) A mixture of a compound having alkyl groups as R¹, R² and R³ and a compound having hydrogen as R² and alkyl groups as R¹ and R³ can be obtained by treating Compound V with alkanol or diazoalkane as mentioned in (1).

In this reaction, a mixture of Compound III having the same alkyl groups R¹, R² and R³ and Compound VI having hydrogen as R² and the same alkyl groups R¹ and R³ can be obtained by using alkanol or diazoalkane which has the same alkane as R¹ of Compound V, and a mixture of Compound III' having the same alkyl groups R² and R³ which are different from an alkyl group R¹ and Compound VI' having hydrogen as R² and different alkyl groups R³ and R¹ from each other can be obtained by using alkanol or diazoalkane which has a different alkane from R¹ of Compound V.

Compound III, Compound III', Compound VI, and Compound VI' each are isolated by separating their mixtures with the use of suitable means.

Compound vi having hydrogen as R² and the same alkyl groups R¹ and R⁴ can be synthesized by hydrolyzing esters of Compound iii as mentioned in (4).

(6) Compound VII having hydrogens as R¹ and R³ and an alkyl group as R² can be obtained by hydrolyzing esters of Compound II with the use of methanol and alkaline at room temperature for several hours as mentioned in (3).

Compound vii having hydrogen as R¹ and R⁴ and an alkyl group as R² can be obtained by mixing Compound i with alkanol such as ethanol and an organic or inorganic acid and by refluxing the mixture for about 1 hour to 2 hours.

(7) Compound II' having hydrogen as R¹ and different alkyl groups R² and R³ from each other can also be obtained by dissolving Compound VII or Compound VIII in an appropriate solvent such as a THF-ether system, adding diazoalkane which has a different alkane from R² or R³ of Compound VII or Compound VIII, and allowing the mixture to stand at room temperature for several minutes to several tens of minutes, or by mixing Compound VII or Compound VIII with alkanol which has a different alkane from R² or R³ of Compound VII or Compound VIII and an organic or inorganic acid, and refluxing the mixture at room temperature for about 1 hour to about 2 hours.

(8) A compound having the same alkyl groups R¹ and R² and a different alkyl group R³ from R¹ and R² can be synthesized by allowing Compound IV to react with diazoalkane or alkanol which has a different alkane from R¹ and R² of Compound IV in the same means as above(7), a compound having the same

alkyl groups R¹ and R³ and a different alkyl group R² from R¹ and R³ can be synthesized by allowing the Compound VI to react as mentioned above, and a compound having different alkyl groups R¹, R², and R³ from each other can also be obtained by treating Compound IV' or Compound VI' with alkanol or diazoalkane which has a different alkane from R¹ and R² of Compound IV' or R¹ and R³ of Compound VI'.

Furthermore, a compound having the same alkyl groups R¹ and R⁴ and a different alkyl group R² from R¹ and R⁴ can be obtained by treating Compound vii with diazoalkane which has a different alkane from R² of Compound vii, or with alkyl iodide which has a different alkyl from R² of Compound vii as mentioned in (2). This compound can also be synthesized by treating Compound vi with alkanol which has a different alkyl from R¹ and R⁴ of Compound vi.

The following describes physicochemical characteristics and structures of RD-01-Compounds methylated in accordance with above alkylation process.

(a) Tetramethyl RD-01-A₃

Molecular Formula: C₂₂H₂₇O₉N

Molecular Weight: 449

SIMS: m/z 450 [M + H]⁺, m/z 472 [M + Na]⁺

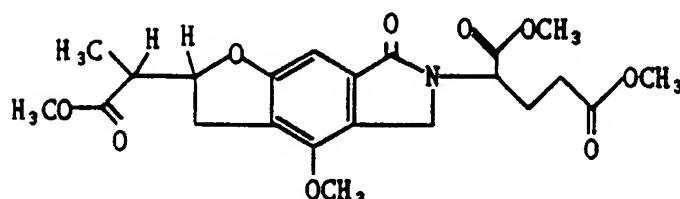
¹H-NMR: (200MHz, CDCl₃) ppm (J = Hz)

1.340(3H, d, J = 7.2); 2.1 - 2.5(4H, m); 2.813(1H, dq, J = 7.2, 7.2); 3.151(1H, dd, J = 7.4, 16.0); 3.498(1H, dd, J = 9.3, 16.0); 3.618(3H, s); 3.718(3H, s); 3.726(3H, s); 3.983(3H, s); 4.317(1H, d, J = 16.2); 4.496(1H, d, J = 16.2); 4.9 - 5.1(2H, m); 6.914(1H, s)

¹³C-NMR: (50MHz, CDCl₃) ppm

13.28(q), 25.04(t), 30.68(t), 32.49(t), 44.89(d), 45.11(t), 51.80(q), 51.99(q), 52.45(q), 53.29(q), 58.59(q), 84.16(d), 98.82(d), 119.00(s), 123.56(s), 133.81(s), 151.16(s), 161.79(s), 168.74(s), 171.12(s), 172.81(s), 173.99(s)

Structure:



(b) Trimethyl RD-01-A₄

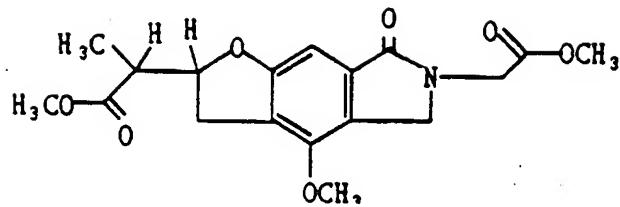
Molecular Formula: C₁₈H₂₁O₇N

Molecular Weight: 363

¹H-NMR: (CDCl₃) ppm (J = Hz)

1.342(3H, d, J = 7.2, 11-CH₃); 2.818(1H, qd, J = 7.2, 7.2, 11-H); 3.138(1H, dd, J = 7.2, 16.2, 3-H); 3.489(1H, dd, J = 9.2, 16.2, 3-H); 4.998(1H, ddd, J = 7.2, 7.2, 9.2, 2-H); 4.378(2H, s); 4.468(2H, s); 6.933(1H, s, 8-H); 3.714(3H, s, COOCH₃); 3.758(3H, s, COOCH₃); 3.971(3H, s, 4-OCH₃)

Structure:



10

(c) Trimethyl RD-01-C1

Molecular Formula: C₁₆H₁₇O₆N

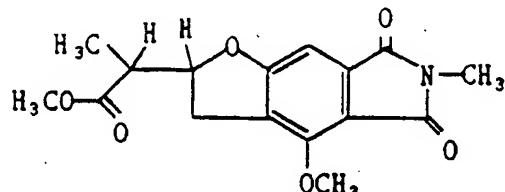
15 Molecular Weight: 319

SIMS: m/z 320 [M + H]⁺, m/z 639 [2M + H]⁺(m/z 320 in HR-SIMS means C₁₆H₁₈O₆N)1H-NMR: (200MHz, CDCl₃) ppm (J = Hz)

20 1.333(3H, d, J = 7.2); 2.825(1H, dq, J = 7.2, 7.2); 3.070(1H, dd, J = 7.6, 16.8); 3.106(3H, s); 3.419(1H, dd, J = 9.6, 16.8); 3.719(3H, s); 4.198(3H, s); 5.098(1H, ddd, J = 7.2, 7.6, 9.6); 6.929(1H, s)

13C-NMR: (50MHz, CDCl₃) ppm 13.04(q), 23.91(q), 31.70(t), 44.83(d), 52.11(q), 61.65(q), 85.51(d), 100.63(d), 112.44(s), 122.33(s), 136.66(s), 154.64, 165.70(s), 166.74(s), 167.72(s), 173.60(s)

25 Structure:



35

(d) Trimethyl RD-01-C2

40 Molecular Formula: C₁₈H₁₉O₈N

Molecular Weight: 377

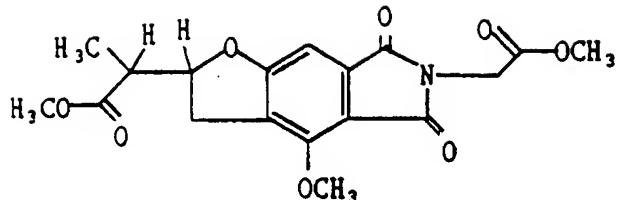
SIMS: m/z 378 [M + H]⁺, m/z 755 [2M + H]⁺1H-NMR: (200MHz, CDCl₃) ppm (J = Hz)

45 1.340(3H, d, J = 7.0); 2.837(1H, dq, J = 7.0, 7.0); 3.088(1H, dd, J = 7.8, 16.8); 3.447(1H, dd, J = 9.8, 16.8); 3.731(3H, s); 3.761(3H, s); 4.210(3H, s); 4.382(2H, s); 5.125(1H, ddd, J = 7.0, 7.8, 9.8); 6.972(1H, s)

13C-NMR: (50MHz, CDCl₃) ppm

50 13.45(q), 32.17(t), 39.20(t), 45.26(d), 52.58(q), 53.07(q), 62.12(q), 86.05(d), 101.43(d), 112.62(s), 123.15(s), 136.82(s), 155.45(s), 166.11(s), 166.46(s), 167.21(s), 168.43(s), 174.01(s)

Structure:



When an agent for inhibiting an aldose reductase of the present invention is administered to patients, the daily dose is 1 mg to 1000 mg, preferably 100 mg to 500 mg, although it depends on sex, age, and the condition of patients. The preferable route of dosage is oral, though either oral or non-oral route can be taken. Agent forms used in the oral administration involves elixirs, capsules, granules, pills, suspensions, emulsions, powders, tablets, and syrups, but tablets are better than the others.

Tablets can be prepared by usual methods for tablets as follows. An aldose reductase inhibitor according to this invention is first rendered granular with or without uniform admixture with a diluent, binder, disintegrator, and other suitable additives. The resultant granules are provided with additives such as a 20 lubricant, and compressed into a desired shape and size. These granules are usually prepared by compressing a drug of the above mixtures and crushing to granules, granulating and drying. Tablets may also be prepared either by direct compression of a drug with or without a diluent, binder, disintegrator, and other suitable additives, or by compression after drugs with or without suitable additives have been added to previously prepared inactive granules. If necessary, coloring agents, flavoring agents, etc. may be added. 25 Tablets may be coated with sucrose or other suitable coating agents.

The following will show the embodiments of the present invention as examples.

Example 1

30 (1) Culturing step

A slant culture of the Crucibulum RF-3817 is used to seed a 500 ml-Erlenmeyer flask charged with 100 ml of a medium which is composed of 20 g of potato starch, 20 g of sucrose, 5 g of yeast extract, and 1000 ml of service water (with an unadjusted pH) and a shake culture is performed at 28°C and 180 rpm for 5 35 days. Four ml of the culture medium is used to inoculate each of one hundred 500 ml-Erlenmeyer flasks charged with 100 ml of a medium which is composed of 20 g of potato starch, 20 g of sucrose, 5 g of yeast extract, and 1000 ml of service water (with an unadjusted pH) and shake cultures are performed at 28°C and 180 rpm for 10 to 15 days.

40 (2) Separating and purifying step

Mycelia are removed from 8 L of the culture medium by filtration. The filtrate (about 8 L) is adjusted to pH 2 by the addition of 2N HCl and then extracted twice with 3 L of ethyl acetate. The ethyl acetate layer is washed with saturated saline and dried by anhydrous sodium sulfate, followed by vacuum distillation, 45 resulting in 4.422 g of crude extract. The crude extract is dissolved in a mixture of acetonitrile and 0.1% TFA in a ratio of 8:2, and the resulting solution is placed on a column of MCI GEL CHP-20P (75 to 150 μ, 200 ml) previously equilibrated with the same mixture. The column is eluted with a concentration gradient consisting of mixtures of acetonitrile and 0.1% TFA in a ratio of 20:80 to 80:20. As a result, fraction 1, fraction 2, fraction 3, fraction 4, and fraction 5 were separated. From fraction 1 were obtained 222 mg of RD-01-A₃ crude product, 954 mg of a mixture of RD-01-A₃ crude product and RD-01-A₄ crude product was 50 from fraction 2, 314mg of RD-01-A₇ crude product was from fraction 3, 467 mg of a mixture of RD-01-C₁ crude product and RD-01-C₂ crude product was from fraction 4, and 1.361 g of RD-01-C₃ crude product was from fraction 5.

55 (a) Isolation of RD-01-A₃ and RD-01-A₄

The mixture obtained from fraction 2 is further separated and purified by chromatography (column: LiCraprep PR-18, 25 to 40 μ, 20φ x 500 mm, solvent: acetonitrile : 0.1% TFA = 13 : 87), resulting in 183

mg of RD-01-A₃ colorless amorphous powder and 145 mg of RD-01-A₄ purified product.

RD-01-A₄ purified product is further recrystallized from 50% isopropanol to yield 77 mg of colorless fine needles.

5 (b) Isolation of RD-01-A₇

The crude product obtained from fraction 3 is further separated and purified by column chromatography (column; LiCraprep PR-18, 25 to 40 μ , 20 ϕ x 500 mm, solvent; (acetonitrile : THF = 8 : 2) : 0.1% TFA = 13 : 87), resulting in 167 mg of RD-01-A₇ purified product. The purified product is further recrystallized from 10 50% methanol to yield 69 mg of colorless fine needles.

(c) Isolation of RD-01-C₁ and RD-01-C₂

The mixture obtained from fraction 4 is further separated and purified by column chromatography (column; LiCraprep PR-18, 25 to 40 μ , 20 ϕ x 500 mm, solvent; acetonitrile : 0.1% TFA = 20 : 80), resulting 15 in 29 mg of RD-01-C₁ purified product and 59 mg of RD-01-C₂ purified product.

RD-01-C₁ product (29 mg) is further separated and purified by HPLC (column; COSMOSIL-5-C18, 20 ϕ x 150 mm, solvent; acetonitrile : 0.1% TFA = 20 : 80) to yield 7 mg of pale yellow amorphous powder.

RD-01-C₂ product (59 mg) is also separated and purified by HPLC (column; COSMOSIL-5-C18, 20 ϕ x 20 150 mm, solvent; acetonitrile : 0.1% TFA = 20 : 80) and resulting purified product (25 mg) is further recrystallized from 50% isopropanol to yield 13 mg of colorless fine needles.

(d) Isolation of RD-01-C₃

The crude product obtained from fraction 5 is further separated and purified by column chromatography (column: LiCraprep PR-18, 25 to 40 μ , 20 ϕ x 500 mm, solvent; acetonitrile : 0.1% TFA = 20 : 80), resulting 25 in 68 mg of RD-01-C₃ purified product. The purified product is further separated and purified by HPLC (column; COSMOSIL-5-C18, 20 ϕ x 150 mm, solvent; (acetonitrile : THF = 8 : 2) : 0.1% TFA = 23 : 77) to yield 12 mg of pale yellow oil.

30 (3) Methylation of RD-01-Compounds

(a) Synthesis of tetramethyl RD-01-A₃

To 0.5 ml methanol solution of 17 mg of RD-01-A₃ purified product obtained in Example 1, 0.2 ml of 35 trimethylsilyldiazomethane (10% n-hexane solution) is added four times every hour (the total amount: 0.8 ml). The resulting mixture is allowed to stand overnight at room temperature, followed by evaporation to dryness after adding a drop of acetic acid. The sample was subjected to TLC (Merck silica gel 60 F254 pre-coated (0.5 mm), toluene and ethyl acetate (1:1) solution system; UV detection). The fraction with an Rf 40 value of 0.4 was extracted with a toluene acetate ester system, resulting in 10 mg of the tetramethyl RD-01-A₃, which was identified by ¹H-NMR, ¹³C-NMR and SIMS as a methyl ether trimethyl ester of RD-01-A₃ obtained in Example 1.

(b) Synthesis of trimethyl RD-01-A₄

To 0.5 ml methanol solution of 5.9 mg of RD-01-A₄ purified product obtained in Example 1, an 45 excessive amount of trimethylsilyldiazomethane (10% n-hexane solution) is added, and the resulting mixture is allowed to stand overnight at room temperature, followed by evaporation to dryness. The sample was subjected to TLC (Merck KGF, toluene and ethyl acetate (1:1) solution system; UV detection). The fraction with an Rf value of 0.3 was extracted with a toluene acetate ester system and evaporated to dryness, resulting in 3.5 mg of the trimethyl RD-01-A₄, which was identified by ¹H-NMR as a methyl ether dimethyl ester of RD-01-A₄ obtained in Example 1.

(c) Synthesis of trimethyl RD-01-C₁

To 1.0 ml methanol solution of 5.5 mg of RD-01-C₁ purified product obtained in Example 1, 1.0 ml of 55 trimethylsilyldiazomethane (10% n-hexane solution) is added, and the resulting mixture is allowed to stand at room temperature for 2.5 hours, followed by evaporation to dryness after adding a drop of acetic acid.

The sample was subjected to TLC (Merck silica gel 60 F254 pre-coated (0.5 mm), toluene and ethyl acetate (1:1) solution system; UV detection). The fraction with an Rf value of 0.6 was extracted with a toluene acetate ester system, resulting in 4.1 mg of the trimethyl RD-01-C₁, which was identified by ¹H-NMR, ¹³C-NMR and SIMS as a methyl ether dimethyl ester of RD-01-C₁ obtained in Example 1.

5

(d) Synthesis of trimethyl RD-01-C₂

To 1.0 ml methanol solution of 7.6 mg of RD-01-C₂ purified product obtained in Example 1, 1.0 ml of trimethylsilyldiazomethane (10% n-hexane solution) is added, and the resulting mixture is allowed to stand 10 at room temperature for 2 hours, followed by evaporation to dryness after adding a drop of acetic acid. The sample was subjected to TLC (Merck silica gel 60 F254 pre-coated (0.5 mm), toluene and ethyl acetate (1:1) solution system; UV detection). The fraction with an Rf value of 0.6 was extracted with a toluene acetate ester system, resulting in 6.3 mg of the trimethyl RD-01-C₂, which was identified by ¹³C-NMR and SIMS as a methyl ether dimethyl ester of RD-01-C₂ obtained in Example 1.

15

Experimental Example

Aldose Reductase-Inhibiting Activity

20 [Procedure]

A homogenate of rat lenses was used as an enzyme source, glyceraldehyde as a substrate, and TPNH as a coenzyme. To a mixture of 0.1 M phosphate buffer (pH 6.2), 0.4 M sodium sulfate, 0.1 mM TPNH (reduced form of triphosphopyridine nucleotide), and 10 mM D, L-glyceraldehyde, a supernatant 25 obtained from the homogenate of rat lenses by centrifugation was added as an enzyme source, so that the change in absorbance at 340 nm became 0.02 units per minute at 25 °C. The change in absorbance at 340 nm for initial one minute was measured at 25 °C, and the inhibiting activity was determined. The results are shown in Table 1.

30

Table 1

	IC ₅₀ (μg/ml)
35	
RD-01-A,	4.7
40 RD-01-A,	0.4
RD-01-A,	1.5
45 RD-01-C,	1.5
RD-01-C,	0.15
50 RD-01-C,	0.7

55

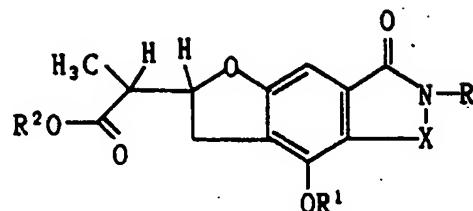
Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. A compound of the formula:

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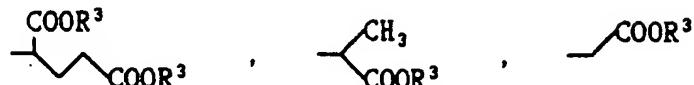
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wherein X is methylene or carbonyl;
 R is

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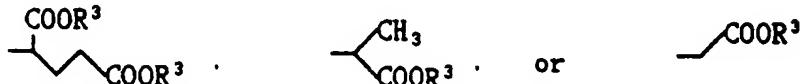
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or R4;
 R1, R2, R3, and R4 are each hydrogen or C1-C4 alkyl, or the salt thereof.

2. The compound of claim 1 wherein R is

30

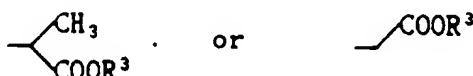
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where X is methylene; or
 R is

R4

40



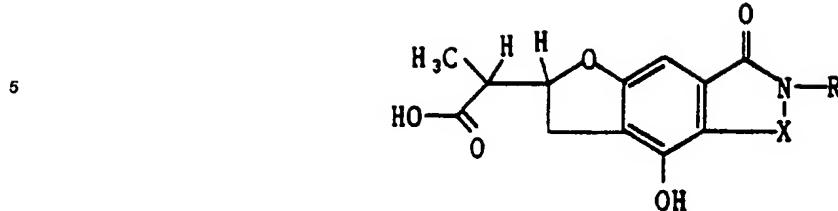
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where X is carbonyl, or the salt thereof.

50 3. The compound of claim 1 wherein R3 or R4 is hydrogen, or salt thereof.

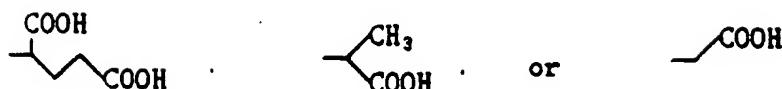
4. A method for producing the compound of claim 1, which comprises culturing Crucibulum sp. RF-3817 or its variants which can produce a compound of the formula:

55



wherein R is

15



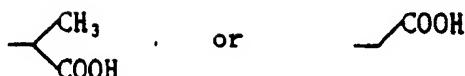
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where X is methylene; or

R is

H,

25



30

where X is carbonyl;

isolating said compound from the cultured medium and optionally alkylating said compound.

35

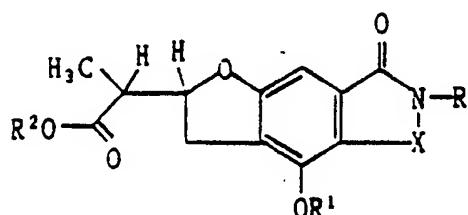
5. A pharmaceutical agent comprising a compound of claim 1, as an effective ingredient, in association with a pharmaceutically acceptable, substantially nontoxic carrier or excipient.
6. The agent of claim 5 which inhibits an aldose reductase.

40

Claims for the following Contracting State : ES

1. A method for producing a compound of the formula:

45

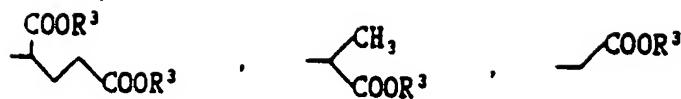


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55

wherein X is methylene or carbonyl;

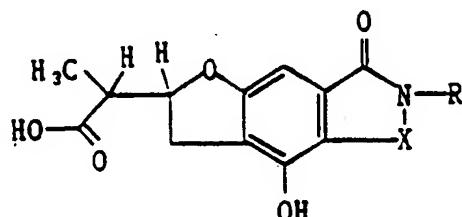
R is



5

or R^4 ;

10 R¹, R², R³, and R⁴ are each hydrogen or C₁-C₄ alkyl, or the salt thereof, which comprises culturing Crucibulum sp. RF-3817 or its variants which can produce a compound of the formula:

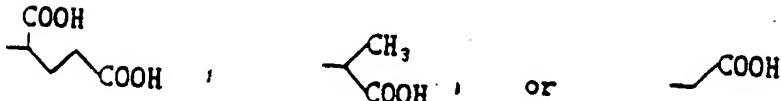


15

20

wherein R is

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30

where X is methylene; or

B is

H

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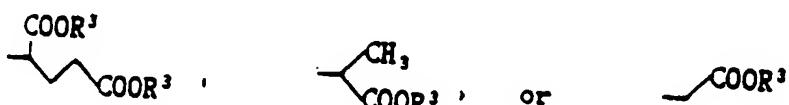


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where X is carbonyl:

isolating said compound from the cultured medium and optionally alkylating said compound.

45 2. The process of claim 1 wherein B is

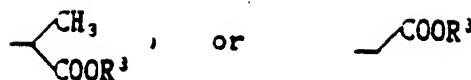


65

where X is methylene; or

WICH

R⁴



where X is carbonyl.

3. The process of claim 1 wherein R^3 or R^4 is hydrogen.

10 4. A process for the preparation of a pharmaceutical agent which comprises combining a compound obtained according to any of claims 1 to 3, as an effective ingredient with a pharmaceutically acceptable, substantially nontoxic carrier or excipient.

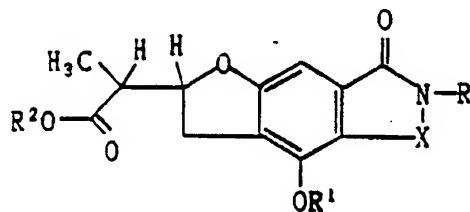
15 5. The process of claim 4 whereby the pharmaceutical agent obtained inhibits an aldose reductase.

Claims for the following Contracting State : GR

1. A compound of the formula:

20

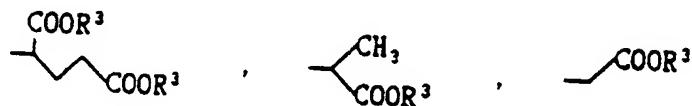
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30

wherein X is methylene or carbonyl;
R is

35



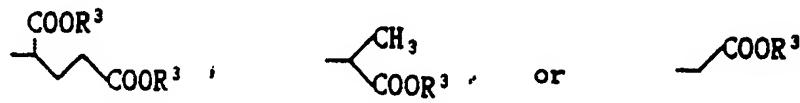
40

or R^4 ;
R^1, R^2, R^3, and R^4 are each hydrogen or C1-C4 alkyl, or the salt thereof.

2. The compound of claim 1 wherein R is

45

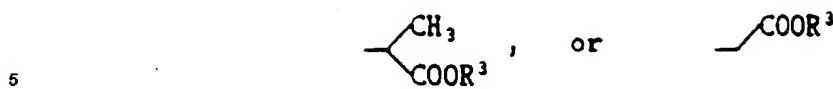
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where X is methylene; or
R is

55

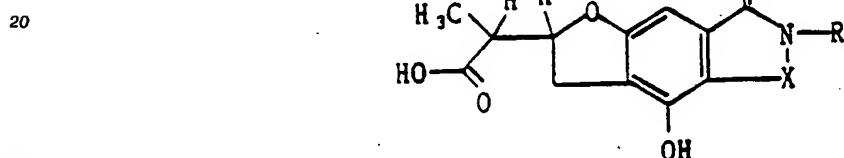
R^4 ,



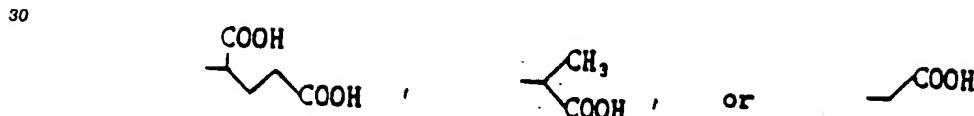
10 where X is carbonyl, or the salt thereof.

15

3. The compound of claim 1 wherein R³ or R⁴ is hydrogen, or the salt thereof.
4. A method for producing the compound of claim 1, which comprises culturing Crucibulum sp. RF-3817 or its variants which can produce a compound of the formula:



30 wherein R is



40 where X is methylene; or
R is

45 H ,



50 where X is carbonyl;
isolating said compound from the cultured medium and optionally alkylating said compound.

5. A process for the preparation of a pharmaceutical agent which comprises combining a compound obtained according to any of claims 1 to 3 as an effective ingredient with a pharmaceutically acceptable, substantially nontoxic carrier or excipient.
- 55 6. The process of claim 5 whereby the pharmaceutical agent obtained inhibits an aldose reductase.

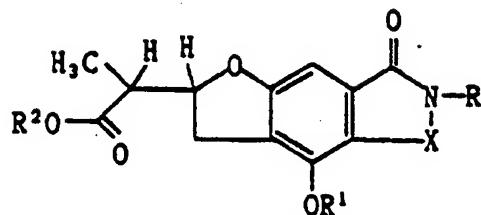
Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Verbindung der Formel

5

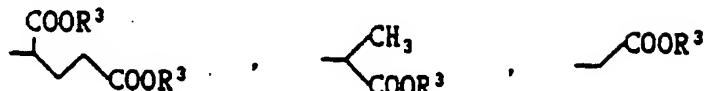
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15

in der X eine Methylen- oder Carbonylgruppe bedeutet;
 R einen Rest der Formeln

20



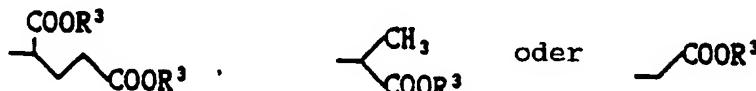
25

oder R⁴
 darstellt; R¹, R², R³ und R⁴ jeweils ein Wasserstoffatom oder einen C₁-C₄-Alkylrest bedeuten, oder ein Salz davon.

2. Verbindung nach Anspruch 1, in der R einen Rest der Formeln

30

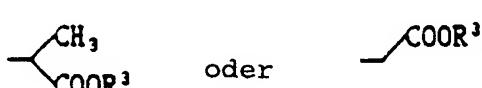
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bedeutet, wobei X eine Methylengruppe darstellt; oder in der R R⁴ oder einen Rest der Formeln

40

45

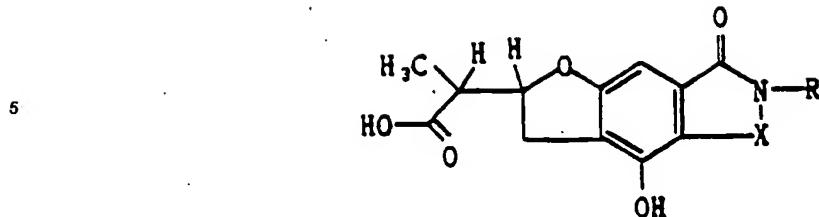


bedeutet,
 wobei X eine Carbonylgruppe darstellt, oder ein Salz davon.

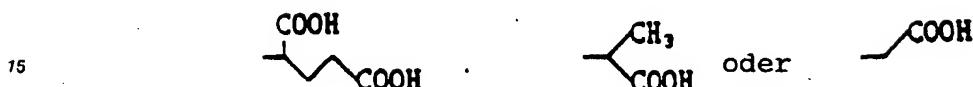
3. Verbindung nach Anspruch 1, in der R³ oder R⁴ ein Wasserstoffatom darstellt, oder ein Salz davon.

4. Verfahren zur Herstellung einer Verbindung nach Anspruch 1, umfassend die Züchtung von Crucibulum sp. RF-3817 oder seiner Varianten, die eine Verbindung der Formel

55



10 herstellen können, in der R einen Rest der Formeln



20 bedeutet,
wobei X eine Methylengruppe darstellt; oder R ein Wasserstoffatom oder einen Rest der Formeln

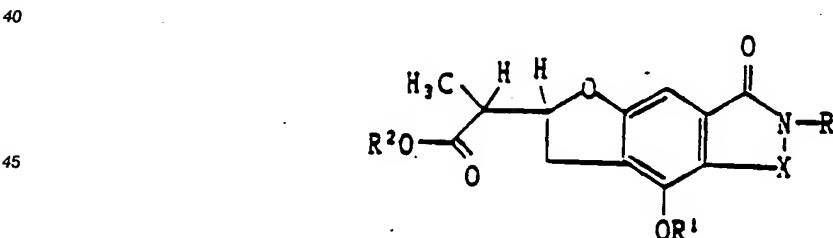
25 bedeutet,
wobei X eine Carbonylgruppe darstellt;
die Isolierung der Verbindung aus dem gezüchteten Medium und gegebenenfalls die Alkylierung der
Verbindung.

30

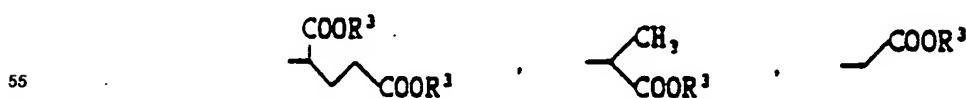
5. Arzneimittel, das eine Verbindung nach Anspruch 1 als Wirkstoff zusammen mit einem pharmazeutisch verträglichen, im wesentlichen nichttoxischen Träger oder Excipienten umfaßt.
6. Mittel nach Anspruch 5, das eine Aldose-Reductase hemmt.

35 Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung einer Verbindung der Formel



45 50 in der X eine Methylen- oder Carbonylgruppe bedeutet; R einen Rest der Formeln

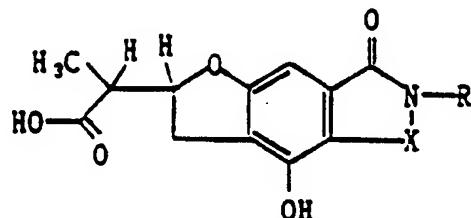


oder R4

darstellt; R¹, R², R³ und R⁴ jeweils ein Wasserstoffatom oder einen C₁-C₄-Alkylrest bedeuten, oder eines Salzes davon, umfassend die Züchtung von Crucibulum sp. RF-3817 oder seiner Varianten, die eine Verbindung der Formel

5

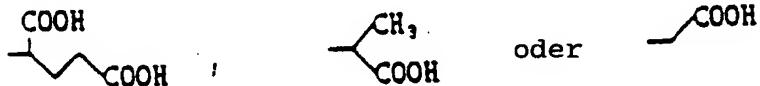
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herstellen können, in der R einen Rest der Formeln

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bedeutet, wobei X eine Methylengruppe darstellt; oder R ein Wasserstoffatom oder einen Rest der Formeln

25



30

bedeutet,

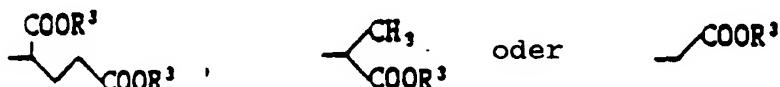
wobei X eine Carbonylgruppe darstellt;

die Isolierung der Verbindung aus dem gezüchteten Medium und gegebenenfalls die Alkylierung der Verbindung.

35

2. Verfahren nach Anspruch 1, wobei R einen Rest der Formeln

40

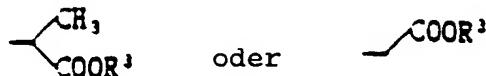


45

bedeutet,

wobei X eine Methylengruppe darstellt; oder R R⁴ oder einen Rest der Formeln

50



55

bedeutet,

wobei X eine Carbonylgruppe darstellt.

3. Verfahren nach Anspruch 1, wobei R³ oder R⁴ ein Wasserstoffatom darstellt.

4. Verfahren zur Herstellung eines Arzneimittels, umfassend die Kombination einer nach einem der Ansprüche 1 bis 3 erhaltenen Verbindung als Wirkstoff mit einem pharmazeutisch verträglichen, im wesentlichen nichttoxischen Träger oder Excipienten.

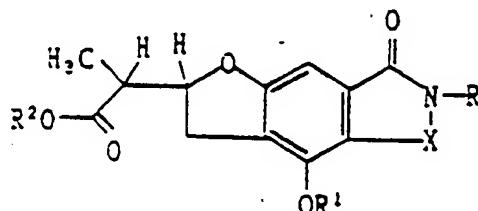
5. Verfahren nach Anspruch 4, wobei das erhaltene Arzneimittel eine Aldose-Reductase hemmt.

Patentansprüche für folgenden Vertragsstaat : GR

1. Verbindung der Formel

10

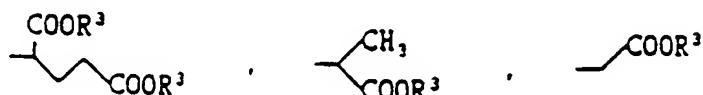
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20

in der X eine Methylen- oder Carbonylgruppe bedeutet;
R einen Rest der Formeln

25

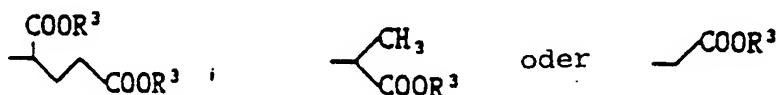


30

oder R4
darstellt; R1, R2, R3 und R4 jeweils ein Wasserstoffatom oder einen C1-C4-Alkylrest bedeuten, oder ein Salz davon.

2. Verbindung nach Anspruch 1, in der R einen Rest der Formeln

35



40

bedeutet, wobei X eine Methylengruppe darstellt; oder in der R R4 oder einen Rest der Formeln

45



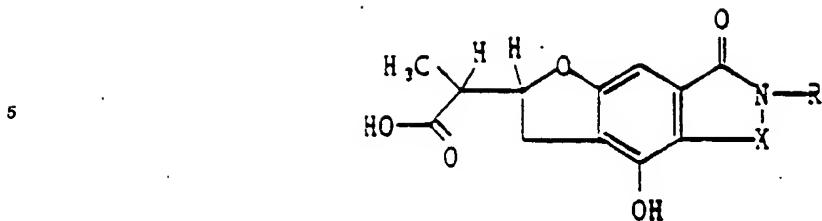
bedeutet,
wobei X eine Carbonylgruppe darstellt, oder ein Salz davon.

50

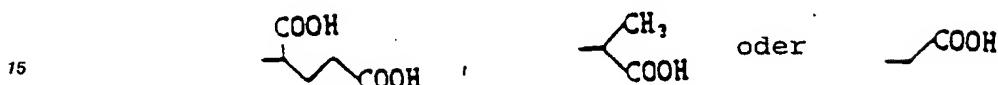
3. Verbindung nach Anspruch 1 in der R3 oder R4 ein Wasserstoffatom darstellt, oder ein Salz davon.

4. Verfahren zur Herstellung einer Verbindung nach Anspruch 1, umfassend die Züchtung von Crucibulum sp. RF-3817 oder seiner Varianten, die eine Verbindung der Formel

55



10 herstellen können, in der R einen Rest der Formeln



20 bedeutet,
wobei X eine Methylenegruppe darstellt; oder R ein Wasserstoffatom oder einen Rest der Formeln



25 bedeutet,
wobei X eine Carbonylgruppe darstellt;
die Isolierung der Verbindung aus dem gezüchteten Medium und gegebenenfalls die Alkylierung der Verbindung.

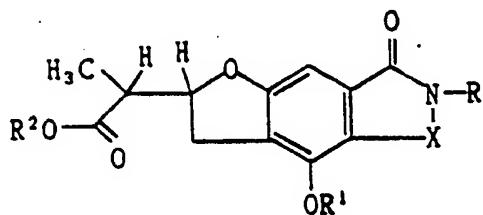
30 5. Verfahren zur Herstellung eines Arzneimittels, das die Kombination einer nach einem der Ansprüche 1 bis 3 erhaltenen Verbindung als Wirkstoff mit einem pharmazeutisch verträglichen, im wesentlichen nichttoxischen Träger oder Excipienten umfaßt.

35 6. Verfahren nach Anspruch 5, wobei das erhaltene Arzneimittel eine Aldose-Reductase hemmt.

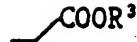
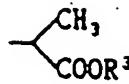
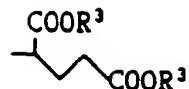
Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

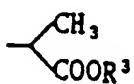
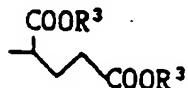
40 1. Un composé de formule :



dans laquelle X est un méthylène ou un carbonyle ;
R est

ou R⁴R¹, R², R³ et R⁴ sont chacun un hydrogène ou un alkyle en C₁-C₄, ou un sel de celui-ci.

10 2. Le composé de la revendication 1 où R est



ou

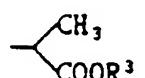


et où X est un méthylène ; ou

R est

R⁴,

20



ou



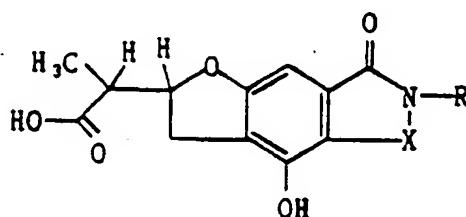
et où X est un carbonyle, ou un de ses sels.

30 3. Le composé de la revendication 1 où R³ ou R⁴ est un hydrogène, ou un de ses sels.

30

4. Un procédé pour la préparation du composé de la revendication 1, qui comprend la culture de Crucibulum sp. RF-3817, ou de ses variantes, pouvant produire un composé de formule :

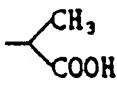
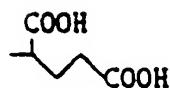
35



40

où R est

45



ou



et où X est un méthylène ; ou

R est

H,

55



et où X est un carbonyle ;
l'isolement dudit composé à partir du milieu de culture et, facultativement, l'alkylation dudit composé.

10 5. Un agent pharmaceutique comprenant un composé selon la revendication 1 en tant qu'ingrédient actif, en association avec un véhicule ou excipient pharmaceutiquement acceptables et essentiellement non toxiques.

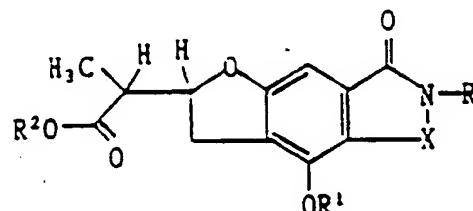
6. L'agent de la revendication 5 qui inhibe une aldose réductase.

15 **Revendications pour l'Etat contractant suivant : ES**

1. Un procédé pour la préparation d'un composé de formule :

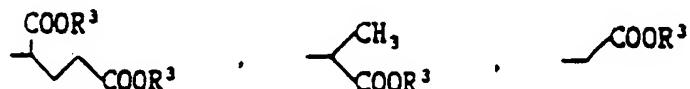
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30 dans laquelle X est un méthylène ou un carbonyle ;
R est

35

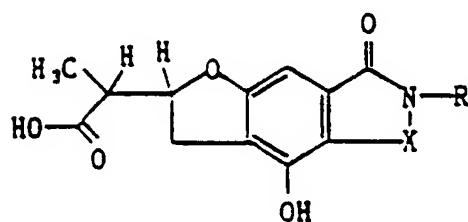


ou R⁴

40 R¹, R², R³ et R⁴ sont chacun un hydrogène ou un alkyle en C₁-C₄, ou un sel de celui-ci, qui comprend la culture de Crucibulum sp. RF-3817, ou de ses variantes, pouvant produire un composé de formule :

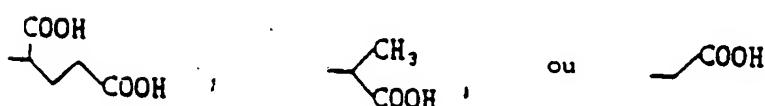
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50



où R est

55



et où X est un méthylène ; ou
R est
H,

5

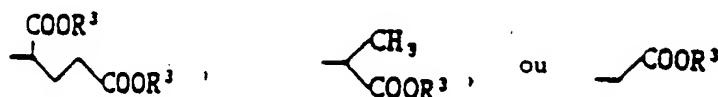


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et où X est un carbonyle ;
l'isolement dudit composé à partir du milieu de culture et, facultativement, l'alkylation dudit composé.

2. Le procédé de la revendication 1 où R est

15



20

et où X est un méthylène ; ou
R est
R4,

25



30

et où X est un carbonyle.

3. Le procédé de la revendication 1 où R³ ou R⁴ est un hydrogène.

35

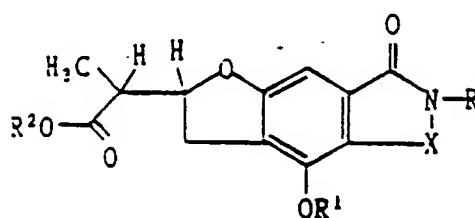
4. Un procédé pour la préparation d'un agent pharmaceutique comprenant la combinaison d'un composé obtenu selon l'une quelconque des revendications 1 à 3, en tant qu'ingrédient actif, avec un véhicule ou excipient pharmaceutiquement acceptables et essentiellement non toxiques.

40 5. Le procédé de la revendication 4, où l'agent pharmaceutique obtenu inhibe une aldose réductase.

Revendications pour l'Etat contractant suivant : GR

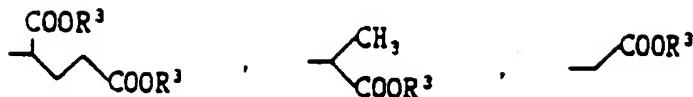
1. Un composé de formule :

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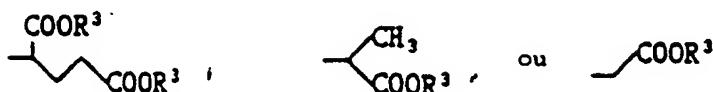


55

dans laquelle X est un méthylène ou un carbonyle ;
R est

ou R⁴R¹, R², R³ et R⁴ sont chacun un hydrogène ou un alkyle en C₁-C₄, ou un sel de celui-ci.

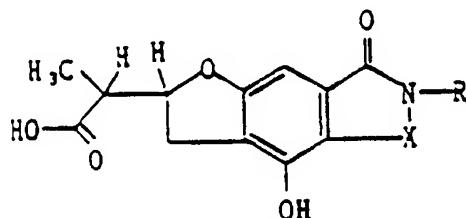
10 2. Le composé de la revendication 1 où R est

et où X est un méthylène ; ou
R est20 R⁴,

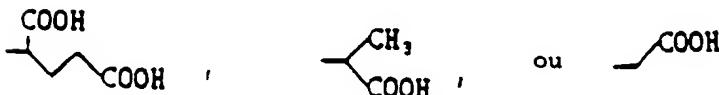
et où X est un carbonyle, ou un de ses sels.

30 3. Le composé de la revendication 1 où R³ ou R⁴ est un hydrogène, ou un de ses sels.4. Un procédé pour la préparation du composé de la revendication 1, qui comprend la culture de Crucibulum sp. RF-3817, ou de ses variantes, pouvant produire un composé de formule :

35



45 où R est

et où X est un méthylène ; ou
R est

55 H,



5

et où X est un carbonyle ;
l'isolement dudit composé à partir du milieu de culture et, facultativement, l'alkylation dudit composé.

10 5. Un procédé pour la préparation d'un agent pharmaceutique comprenant la combinaison d'un composé obtenu selon l'une quelconque des revendications 1 à 3, en tant qu'ingrédient actif, avec un véhicule ou excipient pharmaceutiquement acceptables et essentiellement non toxiques.

6. Le procédé de la revendication 4, où l'agent pharmaceutique obtenu inhibe une aldose réductase.

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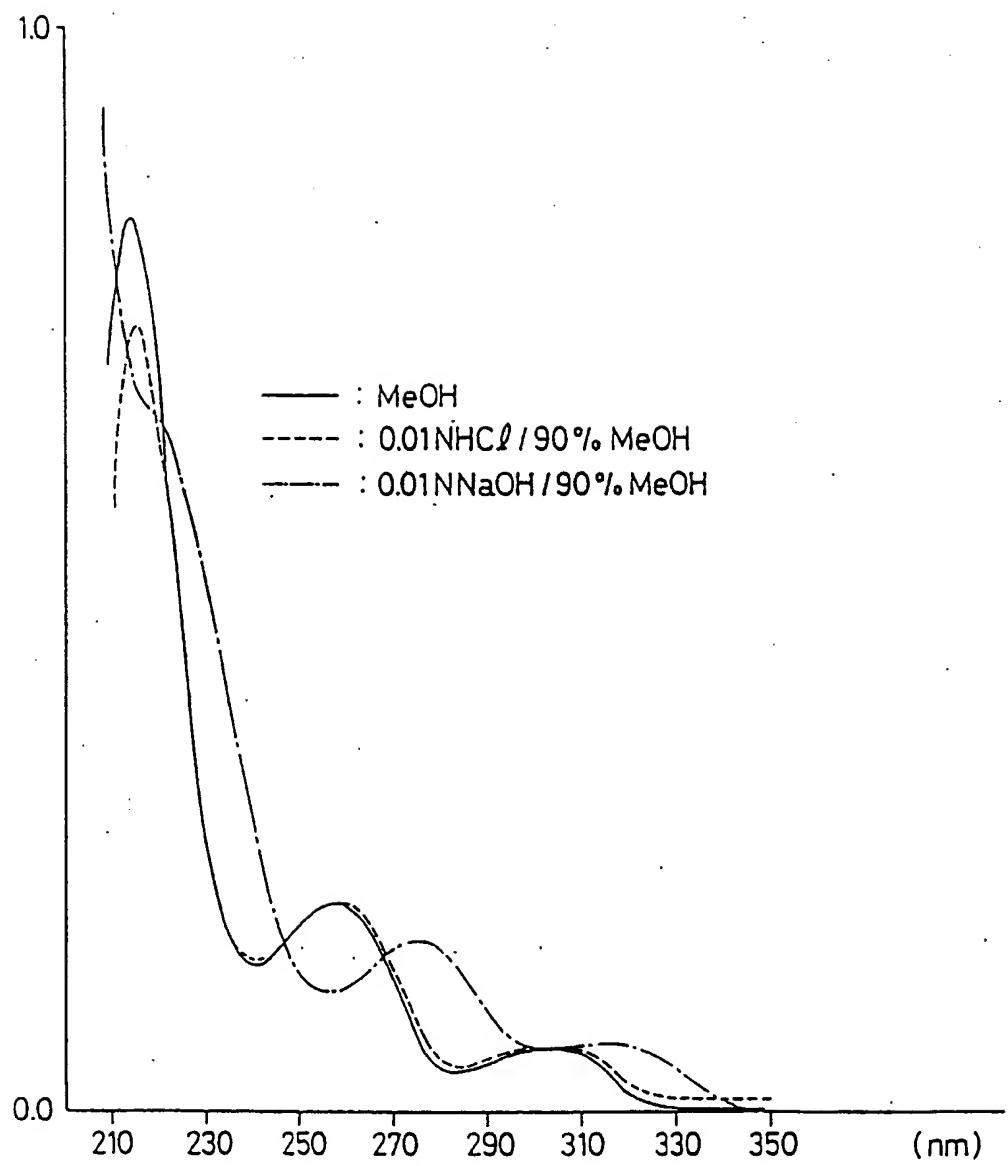
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Fig. 1



EP 0 459 449 B1

Fig. 2

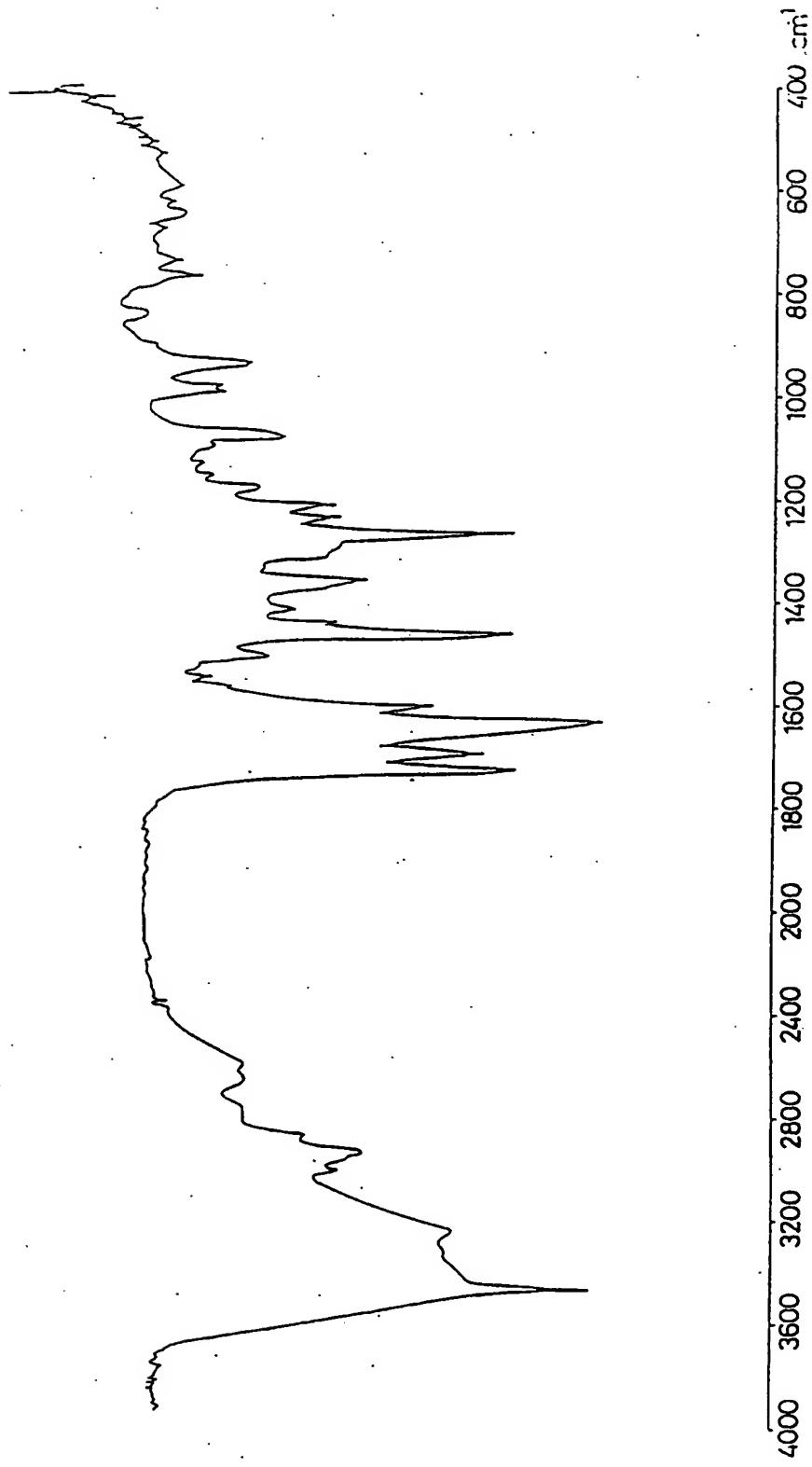
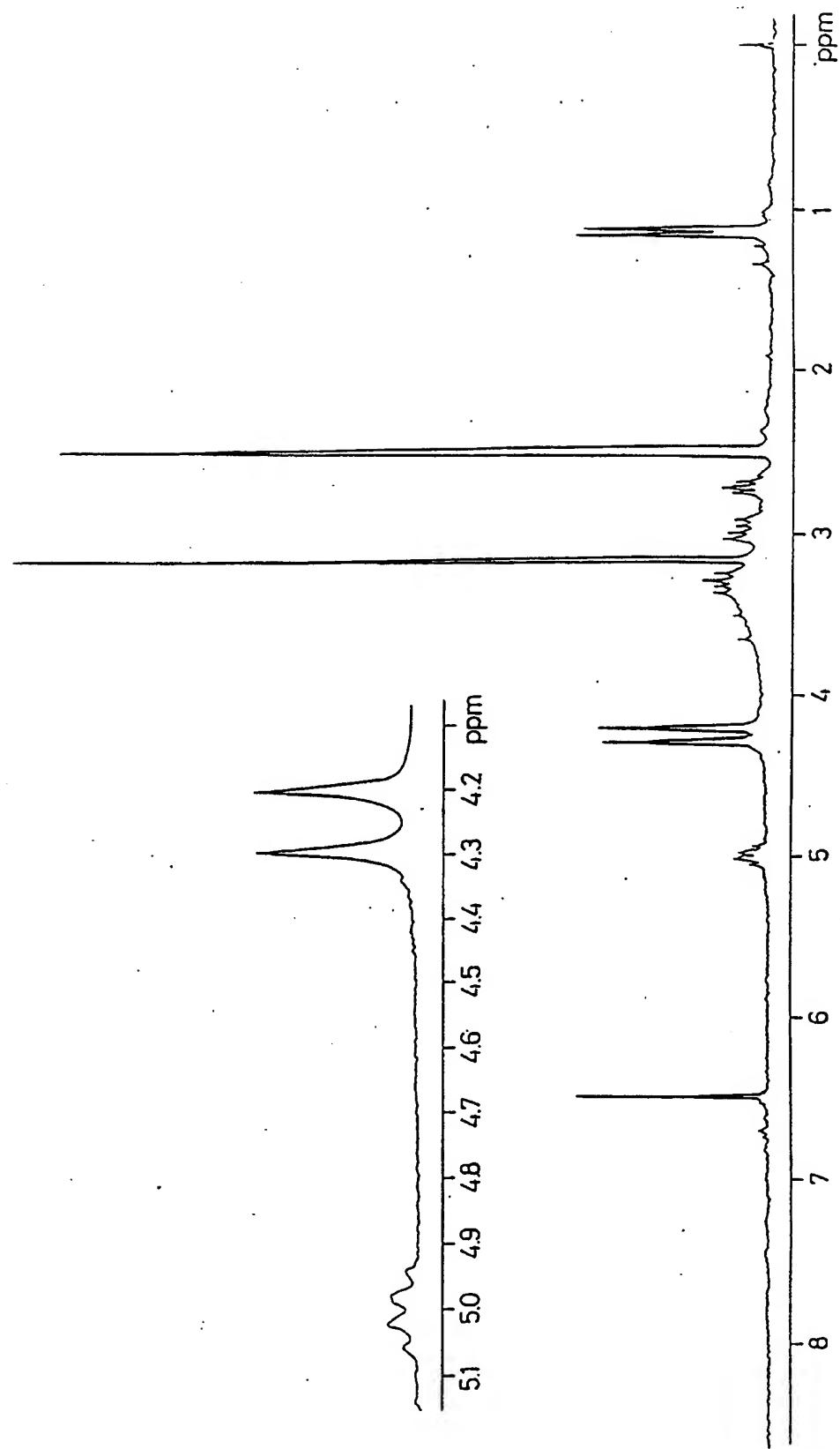


Fig. 3



EP 0 459 449 B1

Fig. 4

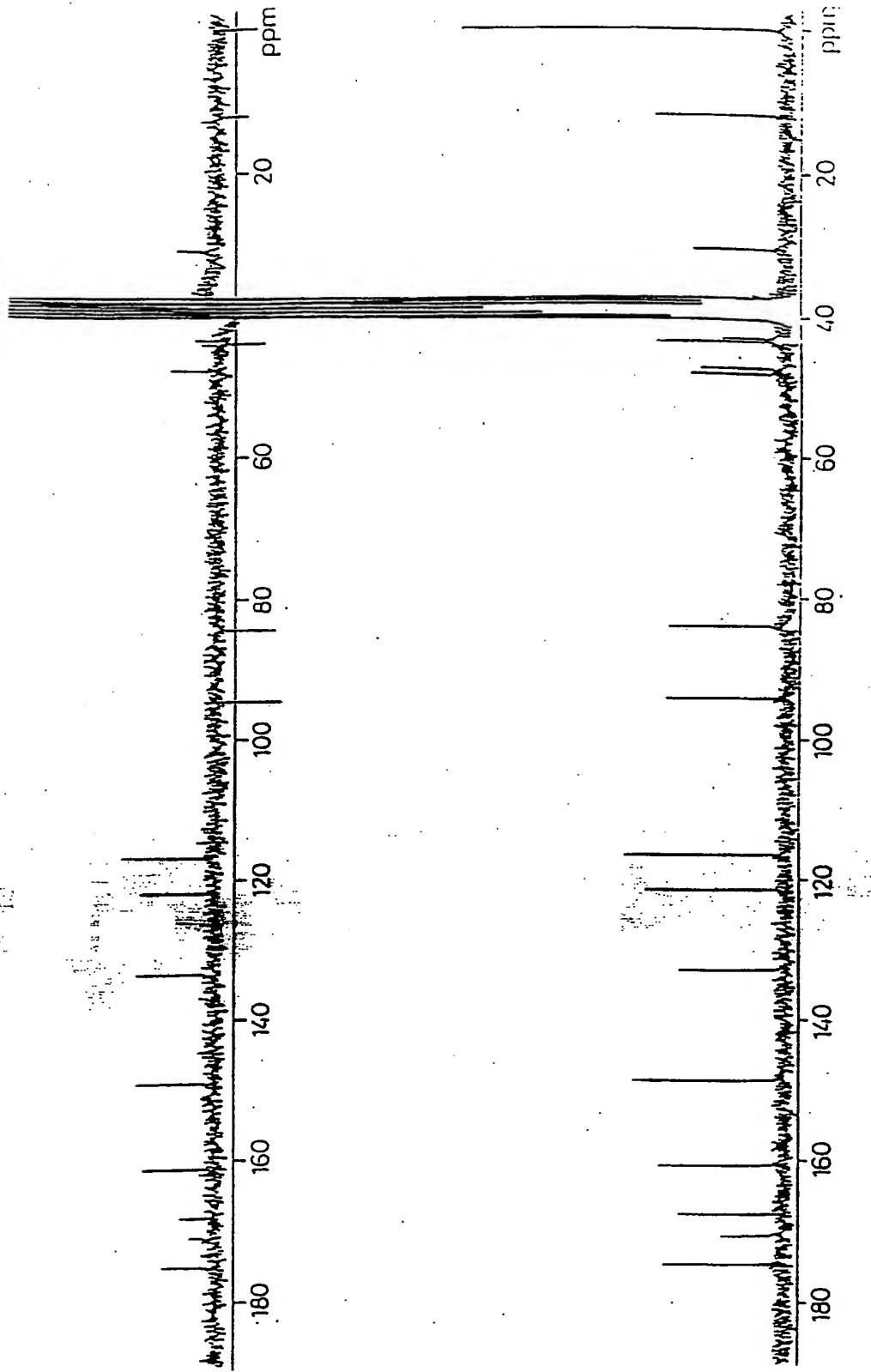
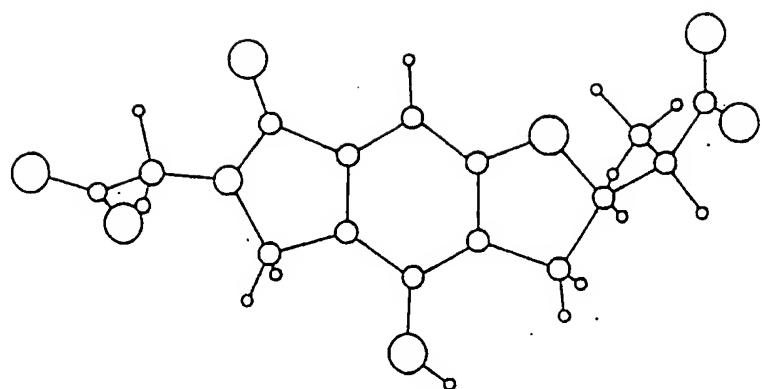


Fig. 5



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